CHEMICAL DATA ON HUMAN EPIDERMAL KERATINIZATION AND DIFFERENTIATION*

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The most important function of the skin is to maintain the integrity of the surface and to protect the organism. Essentially these functions are performed in two ways: by passive (physical) and by active (chemical) means. The structures carrying out these functions have the two basic shapes of elementary units in organisms. Passive protective functions are performed by fibrous or rodlike particles which are capable of close apposition, great rigidity and resistance because of secondary cross-linkages. For chemical defensive measures round or globular particles are best suited with their relatively large surfaces and spaces between the individual units to facilitate the exchange of water and of metabolites.

Both types of structures are represented in the epidermis. Unna noted this duality of epidermal units when he divided the epidermal proteins into the fibrillary spongioplasma and the amorphous granoplasma (1). In our times, Unna's spongioplasma has been replaced by Selby's tonofilaments (2), submicroscopic fibrils present in all layers of the epidermis which are the presumed keratin precursors or prekeratin; while the granoplasma is regarded as an array of cytoplasmic proteins, nucleoproteins and lipoproteins in the epidermal cells. As the horny layer is reached, prekeratinous fibers become consolidated and converted into keratin, while the globular particles are decomposed and carried onto the surface. Upon these two conversions then rests the development and integrity of the horny layer.†

In this work the consolidation of fibrous keratin precursors is called keratinization, while the decomposition of cellular proteins or the formation of non-keratinous components in the horny layer is referred to as differentiation. This distinction is far from academic. Pathologic processes or chemical agents may primarily affect one or the other process. A rational interpretation of epidermal disturbances is impossible, unless the two groups of epidermal components and their fates are studied individually. In Rothman's words, "it is regrettable that the terms 'keratin' and 'keratinous structures' are often used . . . as if they were synonymous" (3).

Important as the distinction between "epidermal keratinization" and "epidermal differentiation" may be, it is still rather artificial. An epidermal cell is a unit; its two basic functions should not be arbitrarily separated. There must be numerous instances and points of convergence where these two processes influence each other. Evidence of such possible interrelation in psoriasis was recently obtained by Anderson Roe. Nevertheless, we cannot hope to understand interrelations, until we know more of the individual processes; hence the necessity for this didactic division between keratinization and differentiation.

Since the appearance of Rothman's publications on these topics (3 to 7), some of our concepts of epidermal development have changed radically. This brief review deals with some important recent advances in the field.

A few words are in order on the terminology adopted. The words "keratinization" to denote the consolidation of keratin precursors and "differentiation" to identify the formation of non-keratinous components in the horny layer are not universally accepted. Other terms for "differentiation", such as "degeneration", "decomposition" or "degradation" may be equally well or even better suited for labeling this process. The difficulty of finding a proper name for epidermal development has been admirably discussed by H. Pinkus in Rothman's textbook (8). It is my belief that controversies over semantics are generally fruitless. The word "differentiation" is chosen here as a fairly neutral term and in the

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† In their latest review, Shelley and Arthur (Physiol. Rev., **20:** 179, 1958), while discussing the "keratinocyte", make the following statement: "The physiology of the keratinocyte is basically the story of the holocrine secretion of a fibrous protein." This statement is erroneously attributed to me. Neither the term "keratinocyte", nor the above "story" reflect my thinking in any way.

hope that it will be replaced soon by more exact physical and chemical terminology.

KERATINIZATION

The study of human epidermal keratinization represents unusual experimental difficulties. The epidermis in most regions is as thin as onion skin paper; its weight is about 3 to 10 mg/cm.sq., depending on the thickness of the horny layer. There have been numerous attempts to overcome these difficulties by analyzing the thick epidermis from specialized areas in animals. Such thickened epidermis is found in the horse burr (9) and the cow's nose (10). Previous reports have not always clearly stated that analytical data, obtained from these animal specimens, are not directly applicable to human epidermis. The histology and composition of horse burr are different from human epidermis (28); epidermin, the fibrous protein isolated from the cow's nose cannot be extracted from human skin (11, 12). Still, analyses of epidermis from the cow's nose furnished unexpected information: epidermin, the fibrous component, has considerably less sulfur than the globular component. This fibrous protein underwent increasing consolidation as it ascended toward the surface (10).

In order to study the source and fate of epidermal keratin, one would have to isolate and analyze the keratin forming cells and compare them with their product, the horny layer. Such isolation has not yet been achieved. Separation of dried pulverized human epidermis into cellular and keratinous fractions by differential centrifugation leaves much to be desired (13). The most promising approach by Szakall makes use of the stripping technique. By removing successive layers from the skin surface with Scotch tape, it is possible to reach the barrier at the base of the horny layer. This barrier which roughly corresponds to the lowermost horny layers can be separated as a coherent membrane and subjected to analyses (14). The painstaking task of studying individual layers within the stratum corneum by such a method has also been attempted (15).

The most practical approach to the chemical study of epidermal keratinization is to analyze the epidermis from an area where the horny layer is thin, such as the abdomen and to compare the results with chemical data obtained in the horny layer. The horny layer may be scraped from hairless skin surfaces to avoid its contamination with lanugo hairs. Stripping the surface with Scotch tape is also a common procedure for collecting experimental material. Calluses have the advantage of yielding relatively large amounts of material, but the objection that callus may be an abnormal horny layer cannot be ignored. Moreover, as it originates from the palms or soles, it probably represents a specialized horny layer which is not characteristic of the major part of the skin surface.

Great caution is indicated when interpreting data in the literature. All too often "epidermal keratin" or "epithelium" is equated with pathologic horny layers, such as scales from exfoliative dermatitis. A handbook even recommends the use of pathological scales for the analysis of epidermis (16). Frequently no distinction is made between "epidermal keratin" and "horny layer", although the two terms are not identical.

When Rothman published his textbook in 1954, there were no complete chemical data of the amino acid composition of human epidermis. In the meantime a complete set of data on the amino acid composition of the stratum corneum and of the epidermis was published by Müting et al. (17). The epidermis was removed from various areas of the body (not from palms or soles) at 50° C.

A comparison of these data with previous values (18) reveals that the amounts of lysine, tyrosine, phenylalanine and methionine are in close agreement with those of a previous investigator. The arginine values are low, even when the 5–10% error inherent in the method (17) is taken into account. Even more surprising are the extremely high values for cystine; they are almost three times the values found earlier. The concentrations of the other amino acids are the only quantitative estimations available and require further confirmation (Table I).

There is only one list of the amino acids of non-pathologic horny layer. These data, by Müting (17), from palmar calluses, are again characterized by unusually low values for arginine and exceedingly high values for cystine. All other estimations (with the exception of the single cystine value for callus by Matoltsy) (19) have been carried out on pathologic specimens or on specimens of doubtful origin (Table II).

When we recall that these determinations have a 5–10% experimental error, it is apparent that the most salient feature of epidermal keratinization is the similarity in the composition of the

TABLE I

Amino acid composition of normal human epidermis

In percent of amino nitrogen

Reference	Source	Arginine	Histidine	Lysine	Tyrosine	Tryptophane	Phenylalanine	Cystine	Methionine	Serine	Threonine	Leucine	Isoleucine	Valine	Glutamic Acid	Aspartic Acid	Glycine	Alanine	Proline	Hydroxyproline
17	Heat separated epidermis	3.6	2.4	5.8	3.4	2.2	5.0	4.8	1.9	9.7	5.3	9.0	3.7	6.6	14.1	5.5	7.8	6.5	4.9	4.5
18	Epidermis separated with 0.1 N acetic acid	6.7	2.6	5.9	4.0	1.0	4.8	1.4	1.6	—	—	—	—	—	—	—	—	—	—	—

TABLE II

Amino acid composition of human horny layers

In percent of amino nitrogen

Reference	Source	Arginine	Histidine	Lysine	Tyrosine	Tryptophane	Phenylalanine	Cystine	Methionine	Serine	Threonine	Leucine	Isoleucine	Valine	Glutamic Acid	Aspartic Acid	Glycine	Alanine	Proline	Hydroxyproline
17	Normal (Callus)	4.3	2.9	7.9	3.7	2.5	7.1	9.1	2.0	8.3	6.0	10.2	2.1	6.7	13.2	4.7	3.7	6.9	6.6	None
19	Normal (Callus)	—	—	—	—	—	—	0.6–0.8	—	—	—	—	—	—	—	—	—	—	—	—
20	Psoriasis	12.2	0.8	4.0	—	—	—	1.9	—	—	—	—	—	—	—	—	—	—	—	—
21	Psoriasis	24.4	1.2	4.7	—	—	—	1.4, 1.8	2.6	—	—	—	—	—	—	—	—	—	—	—
24	Exfoliative dermatitis*	7.9	1.9	7.1	5.0	0.5	2.9	3.5	1.0	17.0	3.5	7.9	3.8	5.4	15.9	8.4	11.4	3.0	3.3	None
25	Exfoliative dermatitis	6.0	0.8	4.3	—	—	—	3.4	—	—	—	—	—	—	—	—	—	—	—	—
26	Not specified	5.9	0.6	4.7	3.4	1.8	—	3.8	—	—	—	—	—	—	—	—	—	—	—	—
	Exfoliative dermatitis	10.0	0.6	3.0	5.7	1.5	—	2.3	—	—	—	—	—	—	—	—	—	—	—	—

* The origin of this horny layer is doubtful. Block refers to it as "epidermis"; however, the values are identical with his less complete data for "epithelium", consisting of scales from a mild case of exfoliative dermatitis (23). Upon my inquiry, Dr. Block kindly informed me that the data came from epidermis separated at 58°C or from scales after sunburn erythema. The lack of hydroxyproline which Müting et al could not explain (17) indicates that this is a specimen of horny layer.

epidermis and the horny layer. There are only two definitely established changes occurring in the course of epidermal keratinization: 1. The disappearance of hydroxyproline and 2. a variable and inconstant rise in cystine. In view of the fact that among 19 amino acids only two have been shown to undergo quantitative changes in the course of epidermal keratinization, it is legitimate to conclude that keratinization essentially represents a rearrangement of the existing building stones of a precursor.

Cystine and cysteine are crucial in keratinization. Their functions and roles have been investigated again and again; yet, we are still uncertain how they fit into the overall picture.

We definitely know that epidermal keratin has

less cystine than other keratins, for example hair or nail. The disulfide groups of cystine bridge the neighboring polypeptide chains in the keratin molecule, thus greatly promoting the stability of the horny layer and its resistance against some chemical influences. These are well established facts. What we do not know is the exact relationship between the sulfhydryl bearing cysteine and the disulfide containing cystine in the various layers of the epidermis.

The classical concept, advanced by Giroud and his coworkers (9), was based on non-specific histochemical methods for sulfhydryl groups and on chemical analyses of an animal specimen, the horse burr, a rudimentary toe, phylogenetically a hard keratinous structure. These authors proposed that epidermal keratinization had the following features:

1. Sulfhydryl groups were limited to the cellular layers of the epidermis; none occurred in the horny layer.

2. In keratinization of hard structures, such as hair, a band of intense sulfhydryl reaction appears immediately underneath the zone of full hardening. This zone was called the keratogenous zone. It was claimed that this zone occurred in hard keratinous structures only and was absent in the epidermis. For this reason the name "phanerogenous" zone was suggested.

3. Since sulfhydryl was absent in the horny layer, the oxidation of the sulfhydryl groups was believed to account for the cystine present in the horny layer.

None of these findings and assumptions could be confirmed by more recent work.

1. Sulfhydryl is not limited to the cellular layers of the epidermis. Although in small quantities, sulfhydryl occurs in the horny layer as well, as shown by specific histochemical and direct chemical tests (27 to 30). It is of considerable interest that the sulfhydryl compounds can be extracted from the horny layer with detergents or with water (31, 32). In some pathologic horny layers, where these groups are greatly increased, they are attached to water-soluble proteins and hence are not part of the fully hardened keratin molecule.

2. With more specific modern stains the keratogenous zone has been demonstrated not only in the hair matrix, but also in the epidermis (30, 33). The significance of this zone is obscure. It could be due to consolidation and dehydration of the tissue immediately before full hardening sets in. It is more likely that sulfhydryl compounds or enzymes accumulate for the final consolidation of the keratin molecule.

3. The theory that all the cystine in the horny layer originated from the oxidation of sulfhydryl groups in the transitional layers of the epidermis, was not borne out by direct chemical analyses (28). When the skin of the sole was cut in thin layers, parallel to the surface, and microscopically similar sections were pooled and analyzed, it was found that the disulfide content of the Malpighian layer was in the same range as in the horny layer. The sulfhydryl content diminished in the more superficial layers; but this decrease was not sufficient to account for more than a fraction of the disulfide groups of the horny layer. The majority of the disulfide groups must have originated from the Malpighian layer. In our original work Van Scott and myself claimed that the presence of cystine in the cellular layers was proof that a fibrous, cystine containing keratin precursor occurred in the depth of the epidermis. This conclusion was unwarranted. A fibrous keratin precursor may and, most probably, does occur in the Malpighian layer. However, there is no evidence that the cystine is attached to this precursor; it may very well be localized in a non-fibrous component. With histochemical methods disulfide groups have been demonstrated in tonofibrils (30); however, even if this observation is accurate (in view of the difficult evaluation of positive disulfide staining in small structures), this does not necessarily mean that the disulfide is *in* the fibrous unit; it may be deposited *around* it.

The epidermal cystine content and its changes in the different layers are apparently not the same in various body regions. It has been claimed that there is little sulfur in the plantar horny layer (17), but these isolated data are not in agreement with other values. On the other hand, it is likely that when we ascend to the more superficial layers, the disulfide content increases in the epidermis of other body regions (34). This increase cannot be accounted for by assuming an oxidation of cysteine to cystine; even if all the cysteine in the epidermis were oxidized to cystine, the combined –SH + –S–S– sulfur would be less in the cellular layer than in the horny layer.

The most likely explanation for the increase is that there is a selective uptake of sulfur containing amino acids during the final consoli-

dation of keratin molecule. Such a selective uptake presupposes the existence of a relatively sulfur-free keratin-precursor in the cellular layers of the epidermis. A fibrous protein of this nature has been extracted from human epidermis by Roe (35). This fibrous protein, tonofibrin, was isolated exclusively from the cellular layers of human epidermis. It had an X-ray diffraction pattern like alpha keratin, could be oriented and stretched into a beta pattern. It contained no sulfur.* In histologic sections upon extraction an amorphous eosinophilic mass appeared in the epidermis. With continued extraction the intercellular bridges and tonofibrils disintegrated.

All these facts can be best integrated in a working theory which assumes that keratinization is a 2-stage process. The first step is the formation of a fibrous precursor in the deeper cellular layers, probably through hydrogen bonding. This precursor is poor in sulfur and is not as completely solidified as keratin. The second step is the combination of this precursor with a sulfur containing protein. Through this combination the final consolidated keratin molecule emerges with its disulfide bridges originating from this secondary coupling.

This scheme is akin to that proposed by Ward and Lundgren for hard keratinization (36). Recently this process has been directly observed in electron microscopic pictures of wool (37) and in the cortex of the human hair follicle (38). At the level of the keratogenous zone, filaments of 60 to 80 Å diameter became embedded into an electron dense mass. Because of its affinity to osmium, this mass was believed to contain considerable amounts of cystine or cysteine. Above this level the amorphous mass of fully consolidated keratin made its appearance.

In electron microscopic pictures of human epidermis the association of the fibrous and non-fibrous components could not be demonstrated. While Selby interprets her findings in the transitional layers of human plantar epidermis as representing lateral joining up of adjacent filaments (2), Birbeck was unable to observe any such changes in abdominal epidermis (39). Again, regional differences, variations in cystine content, or both, may account for this discrepancy. The inability to see the condensation of fibrous and non-fibrous cellular components in epidermal keratinization may be due to two circumstances: 1. There is much less cystine in epidermal keratin than in hair; hence, presumably, the hardening of the fibrous precursor, i.e. its combination with the sulfur-containing globular protein, is much less pronounced. 2. Epidermal keratin is much more diluted with other cellular components than hair. Almost half of the horny layer consists of substances other than keratin, while hair is composed almost entirely of keratin. Therefore the transformation of the filaments may be submerged among the other cellular constituents.

In all recent work the position of keratohyalin remains unsolved. The name of this substance is most unfortunate; lucus a non lucendo—it is neither keratin nor hyalin. Electron microscopic evidence that it gives rise to keratin fibrils (40) remains unconfirmed. Even in the hair follicle where it becomes elongated by a unique process to birefringent "filaments", it is not considered to be a forerunner of keratin (41). Since it does not give rise to keratin fibers, it is safe to assume that its role is in epidermal differentiation. This belief is shared by many. Its absence in parakeratotic horny layers, such as psoriasis, where epidermal differentiation is anomalous, is further indirect proof that it represents a "by-product" of epidermal keratinization. Simultaneous histological and chemical studies of normal and pathologic, parakeratotic and non-parakeratotic horny layers may shed light on this problem.*

EPIDERMAL DIFFERENTIATION

The decomposition of non-fibrous cellular components leads to a vast variety of end-products. Their study is made difficult by the circumstance that some of these soluble compounds originate from the decomposing cells, while others are produced by the sweat and sebaceous glands.

* We may recall that epidermin, the fibrous prekeratin of the epidermis of the cow's nose has a relatively low sulfur content.

* Various theories on the composition and role of keratohyalin are reviewed by Montagna (30). A detailed study of analogies between the granular and parakeratotic horny layers has been published by Braun-Falco (42). Most recently, Charles and Smiddy, without any evidence, claimed that in cornification the "first visible result . . . is the deposition of keratohyalin on the tonofibrillar network of the granular layer cells." The idea is also expressed that "tonofibrils are . . . not necessarily precursors of . . . keratin in the horny layers, although their presence in the horny scales, as a 'keratin' within a keratin, seems to confer on those layers the ability to undergo α–β transformation" (66). In this reviewer's opinion, the awkward expression "keratin within a keratin" expresses the same theory as the one proposed in the present review.

Analyses of skin surface wipings or of the desquamating layers (stratum disjunctum) include both types of components. On the other hand, extracts of the isolated transitional layers contain solely the end-products of decomposing cells. Between the lower and upper layers of the stratum corneum there is a continuous exchange of soluble components in two opposing streams: the downward seepage of sweat, sebum, externally applied water and chemicals is met by a counterflow of decomposition products and of internal water which ascend rapidly to the surface. The situation is further complicated by the selective absorption or process of ion exchange of some components in the keratinous framework of the horny layer which acts as a chromatographic column. Recently attempts have been made to bridge the gap between the surface and depth of the horny layer by analyzing individual layers which were obtained by consecutive stripping. The data reveal interesting gradients (15).

From a phylogenetic standpoint it is conceivable that these compounds appeared in large quantities in the human epidermis when man lost his furry coat. Together with the development of eccrine sweat glands in the course of the evolutionary trend, the hair loss enabled man to adapt himself to hot climates and to invade the tropics (43). However, the loss of fur exposed his skin surface and made it more vulnerable to physical influences, (especially to sunlight) to water and to chemical agents. To compensate for the lost protection, man developed in the exposed epidermis and in the horny layer an intricate system of defense mechanisms. One of these mechanisms was the filling up of the horny layer with a variety of protective substances. Their functions are as follows (44):

1. Water binding
2. Regulation of the flow of water to surface
3. Maintenance of acid pH
4. Neutralization of acids and alkalies (Buffering ability)
5. Protection against ultraviolet irradiation (purine compounds)
6. Chemical combination with some external agents. (Detoxication)

It is significant that neither fish scales, nor wool or hair contain such protective substances in any appreciable quantities (45). Extracts of these horny products are not hygroscopic and the phylogenetically advanced teleost fishes are said not to have any horny layer at all (46). On the other hand, the amphibious seal has large amounts of –SH groups in its horny layer (47); if, as in the human stratum corneum, these groups are attached to water-soluble proteins, it is likely that in its horny layer this species has developed defenses similar to those of human skin. It must be emphasized that the foregoing is purely speculative and is intended to draw attention to a largely unexplored area of comparative biochemistry and physiology.

TABLE III

Composition of the horny layer

	Stratum disjunctum: (48)	Barrier (44):*
Water-insoluble matter ("Crude keratin")	52–55%	58%
Water	5–10%	—
Ether-soluble	10–22%	—
Water-soluble	19–27%	42%

* Szakall's analysis of the barrier was performed on petroleum ether extracted and dried tissue. A 2.4% lipoid content is quoted by him (44), but it is not clear how this value has been obtained.

Detailed data of the composition of the water-soluble components of the horny layer may be found in Spier's, Pascher's (48 to 53, 15) and Szakall's works (14, 44, 54, 55). Here only a brief summary is given.

The overall composition of the horny layer is represented in Table III. Spier's data were obtained from the stratum disjunctum, the most superficial desquamating layer of the stratum corneum (53); Szakall analyzed the barrier, roughly corresponding to the lowermost horny layers (14, 44).

The concentrations of the various water-soluble components are illustrated in Fig. 1. Foremost are the free amino acids (14, 52, 53); their composition is shown in Fig. 2.

Since Blank's work (56, 57), the hygroscopicity of water-soluble components of the horny layer has received ever increasing attention. From the beginning it was obvious that these compounds were responsible for the water-binding ability of the horny layer. In their absence, calluses become brittle. These compounds are also essential to maintain the normal shedding process. As pointed out by Rothman (3, 6, 7), normal desquamation takes place in the form of invisible dry particles. The horny layer apparently breaks

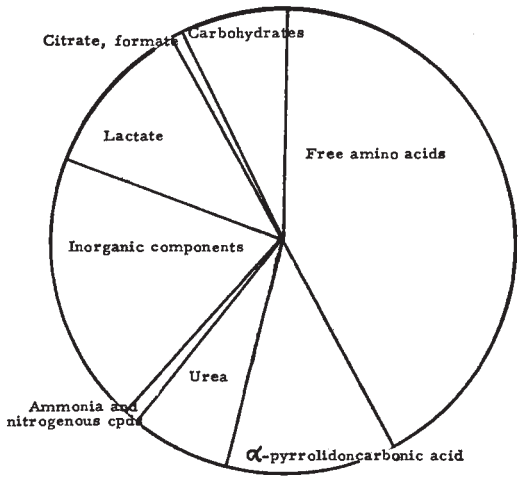

FIG. 1. Over-all distribution of water-soluble components in superficial horny layer (stratum disjunctum) (53).

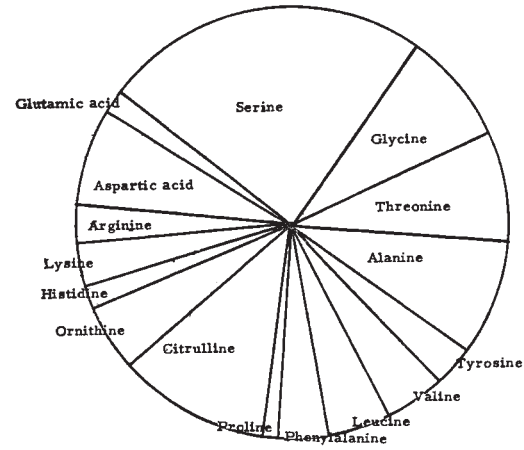

FIG. 2. Distribution of free amino acids in superficial horny layer (stratum disjunctum) (53).

up into individual cells. Under pathologic conditions, the horny layer fails to break up, but forms large coherent scales which stick to each other and to the underlying epidermis. It seems as if a cementing substance between the individual cells were abnormally sticky. This abnormal cohesiveness of psoriatic scales has been noted even under experimental conditions. The scales are hard to break up (58, 59); pulverized pathological scales do not permit the easy flow of liquids through them (59).

Incomplete fragmentation of the horny layer occurs in a large number of conditions. In some as yet not understood way, the break-up of the horny layer and its water-binding capacity may be connected. In some scaling conditions, such as psoriasis or ichthyosiform erythroderma, the water-soluble components hold a great deal less water than the components from normal horny layers (31). Chapping of the skin surface after prolonged use of detergents or in cold weather must have also a chemical basis. The condition may be due 1. to an exhaustive extraction of the water-soluble components when the detergents combine with the epidermal proteins by a simultaneous attack upon the compounds and their lipid shield and 2. to the replacement of the physiologic components with biologically and functionally inferior substances (60). In winter there may be a lesser diffusion to the surface; the decreased insensible perspiration does not replace the lost water-soluble components sufficiently. This impaired restitution is combined with an increased loss from the surface when the skin is inadequately protected by the low sebaceous secretion (3). The dryness of the skin surface in old people may also derive from deficient water-holding components.

The most promising field in epidermal differentiation is the investigation of pathologic horny layers. One of the main bottlenecks in biochemical research of the epidermis is the unavailability of sufficient experimental material. However, scales are usually abundant, easy to handle and to analyze. The remarkable consistency of the concentrations of these compounds has been a source of recurrent amazement to this reviewer, as well as to other authors (14). It suggests a physiological role for these components. Their study has thus provided us with consistent chemical data which have been confirmed. For the first time we are able to attribute definite chemical characteristics to scaling skin diseases. Here only the briefest summary will be given.

In psoriatic horny layers three major anomalies occur: 1. An increase in water-soluble proteins; 2. a decrease in the free amino acid content and 3. an increase in the amount of pentoses.*

1. The increase of the water-soluble proteins is easily observed when we prepare an aqueous

* Anomalies occur also among the lipoid-soluble components. Abnormalities in the metabolism of choline and, much less probably, of cholesterol, have been discussed by Rothman (3). Since the publication of his book, no major advances have been made in this field. The reviewer's omission of the fate of the lipoids during epidermal differentiation is based solely on practical considerations. It should not be interpreted as an attempt to minimize the importance of these compounds in the formation of the horny layer.

extract of pulverized scales and precipitate the proteins by boiling. In extracts of psoriatic scales a heavy white stringy precipitate develops; extracts of callus yield traces only. Actual estimations also show a great increase in the amounts of soluble proteins in the psoriatic horny layer (61, 62). The identity of these proteins is being studied by Roe (61). Here it will be mentioned only that some of these proteins may be precursors of keratin. One of them contains sulfhydryl groups; the high sulfhydryl values of psoriatic scales (27, 28, 29, 59) are linked to this protein.

2. The low free amino acids may reflect a decreased proteolytic enzyme activity in the psoriatic epidermis. Such impaired proteolysis has been demonstrated by chemical (62) as well as histochemical methods (63). The chemical evidence is most unsatisfactory. All the results can be caused by contamination of the specimens with biochemically inert corium by the highly uneven psoriatic dermal papillae. There is no indication that the composition of the amino acids is abnormal; the changes are quantitative, not qualitative (14, 54, 58, 32).

3. The high pentose content of pathologic scales was first described by Szakall (64). Our data equally show the enormous increase of these constituents in the psoriatic scales. Both free and combined pentoses are increased. The role of the pentoses in the horny layer is not definitely known; it has been postulated that they combine with the free amino acids to form hygroscopic compounds in the horny layer (60, 55). The fact that the normal skin of subjects with occupational eczema has abnormally high pentose values (64), indicates that these compounds cannot be simply dismissed as decomposition products.

Such a dismissal would be an evasion of the central issue. The entire horny layer could be properly called a decomposition product. Yet, we have seen how the cells of the epidermis serve the body even in their death. It is impossible not to marvel at the legion of chemical compounds and the multitude of functions in an organ as small as the epidermis. We have barely begun their exploration. It was 4 years ago that Rothman wrote: "In faulty keratinization, disturbances of the cellular decomposition are probably at least as important as anomalies of keratin formation" (3). In the intervening time this statement has been amply justified. It is not unrealistic to predict that the future will bring about an ever increasing knowledge and understanding in this most important field of cutaneous physiology. It is only through such understanding that we can hope for a rational approach to the unsolved problem of scaling skin diseases.*

SUMMARY

Two processes contribute to the development of the horny layer: A. Keratinization, i.e. the consolidation of fibrous precursors of the cellular layers of the epidermis to the fully formed keratin; B. Differentiation, or the decomposition of cytoplasmic and nuclear components to the non-fibrous constituents of the horny layer.

A. Comparison of the amino acid composition of whole human epidermis and of the horny layer indicate that during keratinization the over-all chemical change is slight. The only definitely established differences are the disappearance of hydroxyproline and an inconstant and variable rise in cystine content. The best working theory to account for the changes during keratinization is as follows: Keratinization is a two-step process. The first step is the formation of a fibrous precursor in the cellular layer of the epidermis, with little or no sulfur. This precursor then combines with sulfur containing proteins or protein degradation products to form the consolidated keratin. This combination has been

* In a Festschrift a personal reminiscence may be in order. When I arrived in the United States 16 years ago, brought to this country, as so many others, by Dr. Rothman, he met me in Chicago. I will never forget that meeting. It was a bitter cold March day and the city was pervaded by its famed aroma. While I was shivering in the Arctic blast, Dr. Rothman seemed to be completely unaware of cold and stockyards. With one sweeping gesture of his arm he embraced Billings Hospital, the Midway and the wintry sky. "Isn't this magnificent?" he asked. Then he expanded on the scientific possibilities in this country. "Morphology is a dead end," he said. What we needed were people, trained in basic sciences, to apply their knowledge to exploring the dynamics of skin physiology. Only through an understanding of dynamic processes could we make any progress. It would be presumptuous to state that I understood everything he said. A few days later, however, I heard his first lecture and from then on I knew exactly what he meant. That first lecture dealt with epidermal keratinization. It gives me immense pleasure to think that today, 16 years later, I write on the same topic, extending some of Dr. Rothman's fundamental ideas and that he was able to review these newer concepts in their original version (65).

directly observed in electron microscopic pictures of the hair cortex.

B. The soluble components of the horny layer are essential for maintaining numerous biological functions of the skin surface. This review deals with the water-soluble components. Among them the free amino acids are most important.

In pathologic horny layers these components undergo qualitative and quantitative changes. Psoriatic scales are characterized by increased water-soluble proteins, decreased free amino acids and a high pentose content. Analysis of these soluble components reveals surprisingly constant values, as additional indication of their physiologic role in maintaining the structure and integrity of the horny layer. Further study of these constituents holds great promise for the study of scaling skin diseases.

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ADDENDUM

After completion of this manuscript, Lustig et al. in this journal (**30:** 159, 1958) published complete amino acid analyses of 3 samples of calluses and of scales from psoriasis, exfoliative dermatitis and disseminated neurodermatitis. Their conclusions are in agreement with the views proposed in this review. The analytical data show again the glaring discrepancies between Mueting et al.'s data and those of other authors, especially in respect to arginine and cystine.

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